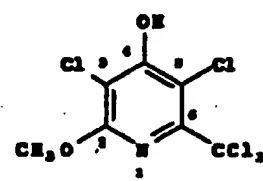




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<p>(21) International Application Number: PCT/US97/09428</p> <p>(22) International Filing Date: 3 June 1997 (03.06.97)</p> <p>(30) Priority Data: 60/019,086 3 June 1996 (03.06.96) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 60/019,086 (CIP) Filed on 3 June 1996 (03.06.96)</p> <p>(71) Applicants (for all designated States except US): THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES National Institutes of Health [US/US]; Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). SOUTHERN RESEARCH INSTITUTE [US/US]; 2000 Ninth Avenue South, Birmingham, AL 35205 (US). JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDECINE [US/US]; 720 Rutland Drive, Baltimore, MD 21205 (US).</p>	<p>(72) Inventors; and (75) Inventors/Applicants (for US only): HARTMAN, Neil [US/US]; 1708 Farragut Avenue, Rockville, MD 20851 (US). STRUCK, Robert, F. [US/US]; 3533 Laurel View Lane, Birmingham, AL 35216 (US). O'REILLY, Seamus [IE/US]; 13230 Falls Road, Hunt Valley, MD 21230 (US). STRONG, John, M. [US/US]; 13103 Bluhill road, Wheaton, MD 20906 (US). ROWINSKY, Eric, K. [US/US]; 14 Dyson Dan Court, Reistertown, MD 21136 (US). COLLINS, Jerry, M. [US/US]; 1512 Auburn Avenue, Rockville, MD 20850 (US).</p> <p>(74) Agents: GAGALA, Bruce, M. et al.; Leydig, Voit & Mayer, Ltd., Suite 4900, Two Prudential Plaza, 180 North Stetson, Chicago, IL 60601-6780 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
(54) Title: PYRIDINE DERIVATIVE, COMPOSITION AND METHOD FOR TREATING CANCER		
<p>(57) Abstract</p> <p>Substantially pure 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine or 4-demethylpenclomedine (formula I), acid addition salts thereof, pharmaceutical compositions containing the aforesaid compound, and a method of using the compound in the treatment of cancer in a mammal are disclosed.</p> <div style="text-align: right;">  <p>(1)</p> </div>		

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PYRIDINE DERIVATIVE, COMPOSITION AND METHOD FOR TREATING CANCERCROSS-REFERENCE TO A RELATED APPLICATION

This is a continuation-in-part of U.S. patent
5 application Serial No. 60/019,086, filed June 3, 1996,
the disclosure of which is incorporated herein in its
entirety by reference.

TECHNICAL FIELD OF THE INVENTION

10 This invention relates to 4-demethylpenclomedine, a
pharmaceutical composition comprising 4-demethyl-
penclomedine, and a method of using the compound in the
treatment of cancer in a mammal.

BACKGROUND OF THE INVENTION

15 It has been estimated that approximately one out of
every three Americans will develop cancer at some point
during life. Currently, in spite of intensive research
and some major advances in treatment, cancer claims the
20 life of nearly one out of every four Americans.

It is indisputable, therefore, that a cure for the
various types of cancer is highly needed. Several cancer
chemotherapeutic drugs are known, for example,
carmustine, doxorubicin, methotrexate, TAXOL®, nitrogen
25 mustard, procarbazine, and vinblastine, to name only a
few.

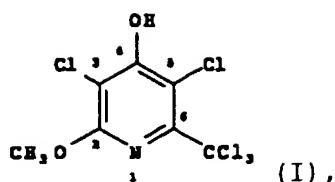
Many of the chemotherapeutic drugs also produce
undesirable side effects in the patient. For example,
U.S. Patent 4,717,726 reportedly discloses a compound
30 suitable for inhibiting the growth of certain types of
malignant neoplasms in mammals. See also Plowman et
al., Cancer Res., 49, 1909-1915 (1989). The disclosed
compound, 3,5-dichloro-2,4-dimethoxy-6-
(trichloromethyl)pyridine, also known as penclomedine, is
35 not satisfactory as a chemotherapeutic, however, because
it is known to produce certain undesirable side effects
especially in the central nervous system.

Neurological and hematological toxicities of penclomedine have been reported in preclinical and early clinical studies. Dose related neurotoxicity, consisting of muscle tremors, incoordination, convulsions and reduced activity, has been observed in rats. Neurotoxicity appears to be related to peak plasma drug concentrations, as it developed during or immediately after infusion and could be ameliorated by decreasing the rate of drug administration. In dogs, severe emesis and seizures have been associated with plasma penclomedine levels above 30 μ M. Neurotoxicity, consisting of dysmetria, ataxia, and vertigo, was also the principal dose limiting toxicity of penclomedine administered as a one hour infusion for 5 consecutive days in patients with advanced solid tumors. The presence of these toxicities, at much lower peak plasma concentrations compared to those reported in preclinical studies, may preclude the administration of higher doses of penclomedine and the achievement of concentrations associated with optimal antitumor activity. Berlin et al., Proc. Amer. Assoc. Cancer Res., 36, 238 (1995); O'Reilly et al., Proc. Amer. Soc. Clin. Oncol., 14, 471 (1995).

Thus, while penclomedine has been tried as an antitumor agent, there remains a need for improved drugs that are effective in combating cancer, but at the same time produce relatively reduced side effects in the patient. An object of the invention, therefore, is to address the above need.

SUMMARY OF THE INVENTION

The present invention provides a substantially pure 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)-pyridine or 4-demethylpenclomedine of the formula (I),



pharmaceutically acceptable salts thereof, pharmaceutical compositions containing the aforesaid compound, and methods of using the compound in the treatment of cancer in a mammal. The compound of the present invention can be in an isolated, purified, or synthetic form.

While the invention is described and disclosed below in connection with certain preferred embodiments and procedures, it is not intended to limit the invention to those specific embodiments. Rather it is intended to cover all such alternative embodiments and modifications as fall within the spirit and scope of the claim.

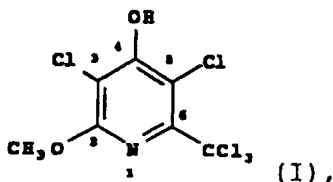
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts levels of penclomedine and the demethylpenclomedine metabolite in human plasma, as determined by HPLC chromatographic analysis with uv detection. These results are for a single patient and are typical. ● = Demethylpenclomedine; ■ = Penclomedine. The displayed curves were fit using a biexponential decay for penclomedine ($\alpha = 3.88 \text{ hr}^{-1}$, $\beta = 0.456 \text{ hr}^{-1}$) and an exponential rise to a constant value for demethylpenclomedine. The Figure shows that penclomedine is rapidly cleared from plasma whereas the metabolite, 4-demethylpenclomedine, continues to increase with time.

Figure 2 depicts the penclomedine (gray shade) and demethylpenclomedine (white) levels in human plasma on each of five treatment days. Data are from a single patient and are typical. The same ordinate scale is used for both penclomedine and demethylpenclomedine. Pre-infusion levels are depicted without cross hatchings; end of infusion levels are depicted with cross hatchings.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a substantially pure
3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)-
5 pyridine or 4-demethylpenclomedine of the formula (I),



pharmaceutically acceptable salts thereof, pharmaceutical
compositions containing the aforesaid compound, and methods
10 of using the compound in the treatment of cancer in a
mammal. The compound of the present invention can be in an
isolated, purified, or synthetic form.

In accordance with one aspect of the present
invention, it has been found that a metabolite of
15 penclomedine, 4-demethylpenclomedine, is surprisingly and
advantageously useful in the treatment of mammalian cancer,
especially human cancer, owing to its potentially reduced
incidence of neurotoxicity, particularly as compared to its
parent compound penclomedine.

20 It has been observed that 4-demethylpenclomedine was
present in clinical samples and continued to be present in
the plasma for longer than 24 hours. Even after repeated
administration of penclomedine, the plasma levels of 4-
demethylpenclomedine was observed to reach greater than ten
25 times the peak plasma level of penclomedine, and thus the
demethylpenclomedine produced drug exposures of several
hundred times that of the parent compound. In fact, the
levels of this metabolite increased as toxicity due to
penclomedine resolved; and on repeated administration of
30 penclomedine neurotoxicity did not increase while levels of
this metabolite increased to several times the plasma
levels of the parent drug. These observations suggest that
the parent drug rather than 4-demethylpenclomedine mediates
neurologic effects.

Penclomedine also is believed to act as a prodrug and thus it needs to be metabolized for it to be active against cancer. Liver function and metabolic rates can affect efficacy. In addition, foods or other drugs present in the
5 body of the mammal during the administration of penclomedine may induce food-penclomedine or drug-penclomedine interactions, which may affect the efficacy of penclomedine.

In keeping with the present invention, 4-
10 demethylpenclomedine can be used alone or in appropriate association, and also may be used in combination with pharmaceutically acceptable carriers and other pharmaceutically active compounds such as other cancer
15 treatment drugs. 4-Demethylpenclomedine also may be used as its acid addition salts. The active agent may be present in the pharmaceutical composition in any suitable quantity.

Examples of pharmaceutically acceptable acid addition salts include those derived from mineral acids, such as
20 hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic, for example p-toluenesulphonic, acids.

25 The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, or diluents, are well-known to those who are skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be
30 one which is chemically inert to the active compounds and one which has no detrimental side effects or toxicity under the conditions of use. The pharmaceutically acceptable carriers can include polymers and polymer matrices.

The choice of carrier will be determined in part by
35 the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the

present invention. The following formulations for oral, aerosol, parenteral, subcutaneous, intravenous, intraarterial, intramuscular, interperitoneal, intrathecal, rectal, and vaginal administration are merely exemplary and
5 are in no way limiting.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets,
10 tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for
15 example, ethanol, benzyl alcohol, propylene glycol, glycerin, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent, or emulsifying agent. Capsule forms can be of the ordinary hard- or soft-shelled
20 gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline
25 cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives,
30 flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and
35 acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

4-Demethylpenclomedine alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized
5 acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

Formulations suitable for parenteral administration
10 include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can
15 include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and
20 related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol such as poly(ethyleneglycol) 400, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, an oil, a fatty
25 acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or
30 carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame,
35 cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl

oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) 5 cationic detergents such as, for example, dimethyldialkylammonium halides, and alkylpyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) 10 nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylene polypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, and (e) 15 mixtures thereof.

The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the active ingredient in solution. Suitable preservatives and buffers can be used in such formulations. In order to minimize or 20 eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5% to about 15% by weight. 25 Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

30 Pharmaceutically acceptable excipients are also well-known to those who are skilled in the art, and are readily available. The choice of excipient will be determined in part by the particular compound, as well as by the particular method used to administer the composition. 35 Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention. The following methods and excipients

are merely exemplary and are in no way limiting. The pharmaceutically acceptable excipients preferably do not interfere with the action of the active ingredients and do not cause adverse side-effects. Suitable carriers and
5 excipients include solvents such as water, alcohol, and propylene glycol, solid absorbants and diluents, surface active agents, suspending agents, tableting binders, lubricants, flavors, coloring agents, and the like.

The formulations can be presented in unit-dose or
10 multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and
15 suspensions can be prepared from sterile powders, granules, and tablets. The requirements for effective pharmaceutical carriers for injectable compositions are well known to those of ordinary skill in the art. See *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Co., Philadelphia, PA,
20 *Banker and Chalmers*, eds., 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., 622-630 (1986).

Formulations suitable for topical administration include lozenges comprising the active ingredient in a
25 flavor, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier; as well as creams, emulsions, gels, and the
30 like containing, in addition to the active ingredient, such carriers as are known in the art.

Additionally, formulations suitable for rectal administration may be presented as suppositories by mixing with a variety of bases such as emulsifying bases or water-
35 soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing,

in addition to the active ingredient, such carriers as are known in the art to be appropriate.

One skilled in the art will appreciate that suitable methods of administering a compound of the present
5 invention to an animal are available, and, although more than one route can be used to administer a particular compound, a particular route can provide a more immediate and more effective reaction than another route.

The present invention further provides a method of
10 treating cancer in a mammal, especially humans. The method comprises administering an effective treatment amount of 4-demethylpenclomedine to the mammal.

As regards these applications, the present inventive method includes the administration to an animal,
15 particularly a mammal, and more particularly a human, of a therapeutically effective amount of the compound effective in the inhibition of neoplasia and tumor growth.

The compound and compositions of the present invention can be administered to treat a number of cancers, including
20 leukemias and lymphomas such as acute lymphocytic leukemia, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's Disease, non-Hodgkin's lymphomas, and multiple myeloma, childhood solid tumors such as brain tumors, neuroblastoma,
25 retinoblastoma, Wilms' Tumor, bone tumors, and soft-tissue sarcomas, common solid tumors of adults such as lung cancer, colon and rectum cancer, breast cancer, prostate cancer, urinary cancers, uterine cancers, oral cancers, pancreatic cancer, melanoma and other skin cancers, stomach
30 cancer, ovarian cancer, brain tumors, liver cancer, laryngeal cancer, thyroid cancer, esophageal cancer, and testicular cancer.

The method of the present invention is particularly applicable in the treatment of brain, colon, renal, and
35 mammary tumors, and preferably colon, renal and mammary tumors. The method of the present invention can be practiced on mammals, particularly humans.

The dose administered to an animal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the animal over a reasonable time frame. One skilled in the art will
5 recognize that dosage will depend upon a variety of factors including the condition of the animal, the body weight of the animal, as well as the severity and stage of the cancer.

A suitable dose is that which will result in a
10 concentration of the active agent in tumor tissue which is known to effect the desired response. The preferred dosage is the amount which results in maximum inhibition of cancer, without unmanageable side effects.

The total amount of the compound of the present
15 invention administered in a typical treatment is preferably between about 60 mg/kg and about 2000 mg/kg of body weight for mice, and between about 5 mg/kg and about 100 mg/kg of body weight and more preferably between 5 mg/kg and about 20 mg/kg of body weight for humans. This total amount is
20 typically, but not necessarily, administered as a series of smaller doses over a period of from about one day to about 24 months, and preferably over a period of 28 days to about 12 months.

The size of the dose also will be determined by the
25 route, timing and frequency of administration as well as the existence, nature, and extent of any adverse side-effects that might accompany the administration of the compound and the desired physiological effect. It will be appreciated by one of skill in the art that various
30 conditions or disease states, in particular chronic conditions or disease states, may require prolonged treatment involving multiple administrations.

The method of the present invention comprises further administering a chemotherapeutic agent other than 3,5-
35 dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine. Any suitable chemotherapeutic agent can be employed for this purpose. The chemotherapeutic agent is preferably

selected from the group consisting of alkylating agents, antimetabolites, natural products, hormonal agents, and miscellaneous agents.

5 Examples of alkylating chemotherapeutic agents include carmustine, chlorambucil, cisplatin, lomustine, cyclophosphamide, melphalan, mechlorethamine, procarbazine, thiotepa, uracil mustard, triethylenemelamine, busulfan, pipobroman, streptozocin, ifosfamide, dacarbazine, carboplatin, and hexamethylmelamine.

10 Examples of chemotherapeutic agents that are antimetabolites include cytosine arabinoside, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, thioguanine, floxuridine, fludarabine, cadribine, and L-asparaginase.

15 Examples of chemotherapeutic agents that are natural products include actinomycin D, bleomycin, camptothecins, daunomycin, doxorubicin, etoposide, mitomycin C, TAXOL (paclitaxel), taxotere, teniposide, vincristine, vinorelbine, mithramycin, idarubicin, MITHRACIN™
20 (plicamycin), and deoxycoformycin.

An example of hormonal chemotherapeutic agent includes tamoxifen. Examples of the aforesaid miscellaneous chemotherapeutic agents include mitotane, mitoxantrone, vinblastine, and levamisole.

25 Demethylpenclomedine can be prepared by any method known to those of ordinary skill in the art. For example, it can be prepared by heating penclomedine in anhydrous dimethylsulfoxide. Thus, a 200 mM solution of penclomedine in anhydrous dimethylsulfoxide (DMSO) can be held at a
30 temperature of about 120-180°C for about 30 minutes to about 2 hours, and preferably at a temperature of about 150°C for about 90 minutes. The principal products are demethylpenclomedine and demethylpenclomic acid. Demethylpenclomedine can be separated and purified by
35 methods known to those of ordinary skill in the art. For example, DMSO can be removed by evaporation under vacuum, the residue dissolved in chloroform, and demethylpenclomic

acid extracted into water. Demethylpenclomedine can be further purified by precipitation from methanol/water. Penclomedine can be prepared by any method known to those of ordinary skill in the art, including that set forth in
5 U.S. Patent 4,717,726. Thus, penclomedine can be prepared by combining 2,3,4,5-tetrachloro-6-(trichloromethyl)pyridine with about two or more molar equivalents of an alkali metal methoxide in an organic solvent under conditions conducive to the formation of 3,5-
10 dichloro-2,4-dimethoxy-6-(trichloromethyl)pyridine as a reaction product, and thereafter, recovering the product.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

15

EXAMPLE 1

This Example illustrates a method of synthesis of 4-demethylpenclomedine.

Penclomedine (6.38 g, prepared from 2-trichloromethyl-
20 3,4,5,6-tetrachloropyridine) in 20 ml of DMSO was heated with stirring to 150°C in 15 min. and maintained at 150-160°C with stirring for 45 min. The reaction solution was frozen and lyophilized, and the residue was stirred with 50 ml of ether. The ether solution was decanted from an
25 insoluble syrup, and the ether solution mixed with 100 ml of hexane. The mixture was allowed to stand at room temperature until the supernate was clear. The supernate was then decanted from the insoluble oil and dried over anhydrous sodium sulfate. The dried solution was decanted
30 from the sodium sulfate and concentrated *in vacuo* with stirring to 20 ml, during which time a crystalline solid precipitated. The precipitate was collected by filtration and dried *in vacuo*: yield, 3.8 g (62%). FABMS analysis: (M+1)⁺310 (5C1); TLC homogeneous on silica gel in methylene
35 chloride: methanol (9:1); TLC after reaction with excess diazomethane indicated essentially complete conversion to penclomedine with a trace of immobile impurity in

hexane:methylene chloride (1:1). ^1H NMR(CDCl_3): δ 4.09 ppm
(Ar-O- CH_3 at 2-position).

EXAMPLE 2

- 5 This Example illustrates the efficacy of penclomedine
and 4-demethylpenclomedine in the treatment of cancer. MX-
1 mammary tumor xenograft was subcutaneously implanted into
mice, and the mice were exposed to the compounds. The
testing of the mice was performed following a published
10 procedure. See, e.g., Plowman et al., supra; Geran et al.,
Cancer Chemother. Rep., Part 2, 1-55 (1972); Developmental
Therapeutics Program, Division of Cancer Treatment, NCI, in
In Vivo Models 1976-1982, NIH Publication No. 84-2635,
Washington DC, U.S. Govt. Printing Office (1984).
- 15 The results obtained are set forth in Table 1 and
confirm that the compound of the present invention is
effective in treating cancer. Although administration of
penclomedine resulted in a slightly higher survival rate,
penclomedine and 4-demethylpenclomedine yielded identical
20 tumor growth delay. Furthermore, penclomedine is
neurotoxic to mammals, especially to humans, whereas
4-demethylpenclomedine is not.

Table 1. Response of Subcutaneously-Implanted MX-1 Mammary Tumor Xenograft to Treatment With Pencloimidine and 4-Demethylpencloimidine (DM-PEN)

Treatment ¹		Tumor Regression ³				
Agent	Dose mg/kg	Route	Schedule	Number of Non-Specific Deaths ² /Total	Partial Complete	Duration ⁴ Med/Range (Days)
					Tumor Free Survival/Total ⁵	Days ⁶ to 2 Doublings
						Days ⁷ Delay (T-C)
Control	0	IP	Q 1 nx5 Day 12		0/10	8.0
Pencloimidine	135	IP	Q 1 nx5 Day 12	0/5	0 5	5/5
						>49.0
Pencloimidine	90	IP	Q 1 nx5 Day 12	0/5	0 5	5/5
						>49.0
Pencloimidine	60	IP	Q 1 nx5 Day 12	0/5	0 5	4/5
						>49.0
DM-PEN	135	IP	Q 1 nx5 Day 12	1/5	0 4	35.7 (35-36)
						>49.0
DM-PEN	90	IP	Q 1 nx5 Day 12	0/5	0 5	32.5 (30-35)
						>49.0
DM-PEN	60	IP	Q 1 nx5 Day 12	0/5	0 2	UE
						23.4
						15.4

10

1. Treatment started on Day 12 following tumor implant.

2. Nonspecific deaths: A treated, tumor-bearing animal was presumed to be a nonspecific death if its day of death was significantly less ($p < 0.05$) than the corresponding day of death in the untreated control group and its tumor was less than 400 mg, or if it died with a tumor of 400 mg or less prior to 45 days after the last day of treatment, or with a regressing tumor prior to 15 days after the last day of treatment, or if the treated animal was uniquely specified as a nonspecific death on data input.

15

5

3. Tumor regression was scored (excluding nonspecific deaths), according to the smallest tumor size attained after the beginning of treatment relative to the size at first treatment:
 - 5 Partial: <50 percent of its size at 1st treatment, but not complete.
Complete: tumor becomes unpalpable.
 4. Duration of regression: the interval during which a tumor classified as a partial or complete regressor was below 50 percent of its size at first treatment.
 - 10
 5. Evaluation size: this value is the tumor mass selected at one or two mass doublings beginning with the initial tumor size at the start of treatment.
 - 15
 6. Time required for tumor mass doubling:
 - the time required for a tumor to double in mass is calculated based on the initial tumor weight at the beginning of the treatment period. When the initial tumor weight has been selected, tumor weights are then examined, beginning with the last recorded value, until a doubling is calculated. Examination from the last recorded value is to insure that the doubling time is calculated during the final phase of tumor growth and not prior to a tumor regression. Values between measurements are calculated by exponential extrapolation, and a value may be estimated after the final measured weight provided the extrapolated value occurs prior to the animal's death.
 7. T-C (days): the difference in the median of times postimplant for tumors of the treated groups to attain as evaluation size compared to the median of the control group. The t-c value is measured excluding nonspecific deaths, tumor-free survivors, and any other animal whose tumor failed to attain the evaluation size.
 - 20
-

EXAMPLE 3

This Example further illustrates the efficacy of penclomedine and 4-demethylpenclomedine in the treatment of cancer. The compounds were tested in vivo on mice by the procedure set forth in Example 2. The results obtained are set forth in Table 2 and confirm that 4-demethylpenclomedine is active against cancer, particularly mammary, colon, and renal cancer. As can be seen from Table 2, the difference in the median of times poststaging for tumors to double in mass of the treated (T) vs. control (C), i.e., T-C, confirms that the compound of the present invention is active against MX-1, colon, and renal tumors. As discussed earlier, although penclomedine produced a slightly higher survival rate than 4-demethylpenclomedine, the two compounds produced nearly identical T-C. These results further confirm the efficacy of 4-demethylpenclomedine in reducing tumor growth.

Table 2. Antitumor Activity of 4-Demethylpenclozidine and Penclozidine

Tumor ^a	Optimal IP Dosage ($< LD_{50}$) (mg/kg/dose)	Schedule (days)	Median %ILS ^c (dying mice only)	T-C ^b (days)	Tumor-Free Survival/Total
<u>4-Demethylpenclozidine</u>					
sc MX-1	90	12-16	—	>41.0	3/5
ic MX-1	90	1-5	+60	—	0/5
ic MX1-1	135 ^{d,e}	1-5	+66	—	1/5
sc MX-1	135 ^{d,e}	13-17	—	36.7	2/5
sc MX-1	90	15-19	—	>37.2	3/5
sc HT29	90	14-18	—	6.0	0/5
sc CAKI-1	90	19-23	—	14.5	0/5
<u>Penclozidine</u>					
sc MX-1	135	12-16	—	>41.0	5/5
ic MX-1	90	1-5	+88	—	0/5
ic MX-1	90 ^d	1-5	+72	—	0/5
sc-MX-1	135	13-17	—	>38.5	5/5
sc MX-1	135	15-19	—	>37.2	5/5
sc HT29	135	14-18	—	1.5	0/5
sc CAKI-1	135	19-23	—	15.3	0/5

^a Athymic mice (Ncr-nu) were implanted either intracranially (ic) with 18 human MX-1 mammary tumor cells or subcutaneously (sc) with fragments of human tumors (CAKI-1, renal; HT29, colon; MX-1, mammary).

^b The difference in the median of time poststaging for tumors to double in mass: T (treated mice), C (control mice)

^c % ILS (increased life span) values are not provided where the mice were sacrificed when tumors reached 4 g mass.

^d Oral treatment (by gavage).

^e Highest dosage level.

EXAMPLE 4

This Example illustrates the methodology employed in the identification and measurement of the distribution of penclomedine and its metabolites in plasma and tissues.

5 CD2F₁ Mice were obtained from Harlan Sprague-Dawley (Frederick, MD). During the course of this study, mice were housed in polyethylene shoe box cages without bedding and given access to food and water. Four mice were used per time point.

10 ¹⁴C-Penclomedine (specific activity 17.6 and 18.9 mCi/mM, labeled at CCl₃) was obtained from Research Triangle Institute (Research Triangle Park, NC) and stored at -20°C. The site of radiolabel has been conserved in all penclomedine metabolites identified to
15 date. The composition of the radioactive material was determined to be 99% ¹⁴C-penclomedine, by HPLC coupled with radiochemical detection.

¹⁴C-penclomedine (1 mCi/ml in ethanol) was added to the clinical penclomedine emulsion to give an activity of
20 40 mCi/ml (4% ethanol final concentration). The mice were administered the above penclomedine formulation at 40 mg/kg (120 mg/m², »100 ml/animal) either intravenously via tail vein or orally via gavage. At 1, 2, 4, and 22 hours after intravenous administration and 2, 4, 6, and 22
25 hours after oral administration, animals were euthanized with carbon dioxide and blood tissues were collected. Blood tissues from each time point were pooled. Blood was centrifuged and plasma and red cells were separated; tissues were homogenized in 3 volumes of 100 mM ammonium
30 formate buffer pH 6.5. Urinary output was estimated by washing the cages with water after removing food and feces and concentrating these cage washings.

A 1 ml aliquot of tissue homogenate or a 200 ml of plasma was acidified with 200 ml of 0.7 M ammonium
35 phosphate pH 2.7, then 3 ml of ethyl acetate was added. This mixture was vortexed, centrifuged and the organic layer was collected. Fifty microliters of DMSO were

added, and the ethyl acetate was concentrated to approximately 100 ml with a stream of dry nitrogen. Fifty microliters of acetonitrile were added to this residue, and the resulting solution was analyzed by HPLC.

- 5 Human plasma was processed identically to mouse plasma with the exception that 500 ml of plasma was extracted. Concentrated cage washings were analyzed by HPLC without further processing.

- 10 Plasma and tissue protein binding data were measured on the residue of the above extractions. The aqueous phase was washed with 5 ml of ethanol, centrifuged, and the ethanol insoluble precipitate was washed again with 5 ml ethanol. After centrifugation the pellet was resuspended in 0.5 ml 6 M guanidinium chloride, added to 15 10 ml 3a70B scintillation cocktail (Research Products International Corp., Mount Prospect, IL), and counted for ^{14}C .

- HPLC Assays: The HPLC system consisted of a Hewlett-Packard Series II 1090 liquid chromatograph with 20 diode array detector (Hewlett-Packard, Palo Alto, CA). The column used was an Alltech Adsorbosphere HS C18 5 m 250 x 4.6 mm column (Alltech Associates, Deerfield, IL). The system used a gradient elution consisting of 100% 10 mM ammonium phosphate buffer pH 2.7 progressing to 100% 25 acetonitrile over 25 minutes at 1 ml/min. Detection was performed by means of ultraviolet absorbance at 240 nm as well as by means of radioactivity using a Radiomatic Flo-One/Beta A140 radioactive flow detector (Packard Instrument Co., Downers Grove, IL) equipped with a 500 ml 30 liquid cells and Flo-Scint VI scintillation cocktail at a 2:1 ratio.

- GC/EI/MS Metabolite Identification: Metabolites were identified by comparing the mass spectra of the incubation extracts with those of synthetic standards. 35 Mass spectra were obtained on a Hewlett Packard 5890 Series II Gas Chromatograph equipped with Model 5971 Mass Selective Detector. Compounds were separated on a 20 m x

0.25 mm i.d. DB-5 fused silica capillary column (Alltech Associates, Deerfield, IL). Helium was used as the carrier gas at a flow rate of 0.6 ml/min; the temperatures of the injector and transfer lines were 200
5 and 270°C, respectively. The column temperature was held at 150°C for 4 minutes after sample injection and then linearly increased to 290°C at a rate of 10°C/min.

Sample Preparation for GC/EI/MS: Plasma and tissue homogenates were extracted with ethylacetate and organic
10 phase evaporated with a stream of dry nitrogen. Samples were either reconstituted in ethylacetate and injected directly (2ml) into the GC or reacted with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Supelco Inc., Bellefonte, PA).

15

EXAMPLE 5

This Example illustrates an advantage of using demethylpenclomedine as a cancer treatment drug in comparison to penclomedine. Particularly, this Example
20 illustrates that demethylpenclomedine does not accumulate in fatty tissues as much as penclomedine.

¹⁴C-Penclomedine derived radioactivity was determined in mouse urine samples collected at 1, 2, 4, and 22 hours following intravenous and oral administration of ¹⁴C-
25 penclomedine. Penclomedine and a number of metabolites could be detected in most mouse tissues as set forth in Table 3. All 2 hour tissue samples contained detectable amounts of penclomedine. There were considerable differences in metabolite distribution among the
30 different tissues. For example, liver contained the highest levels of demethylpenclomedine and the lowest levels of penclomedine. Highest levels of penclomedine were detected in the fat and carcass. Most compounds present in the 2 hour samples were detectable in the 22
35 hour samples at levels from 10% to 50% of those seen at 2 hours.

Table 3. Distribution of Penclomedine and its Metabolites in Mouse Tissues

Tissue	Time (hours)	Tissue Concentration (nmoles/gram wet tissue)							
		Demethyl- Dechloro- Penclomedine ¹		Demethyl- Penclomedine		Dechloro- Penclomedine ²		Penclomedine	
		i.v.	p.o.	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
Liver	2	9.15	8.5	40.5	28.8	n.d.	n.d.	0.69	1.35
	22	1.73	0.63	13.5	5.65	n.d.	n.d.	n.d.	0.38
Kidney	2	4.02	6.10	13.7	13.0	0.39	0.87	6.45	7.30
	22	1.44	0.75	4.89	2.08	n.d.	n.d.	1.21	0.85
Brain	2	1.18	1.10	2.02	3.36	0.26	0.56	1.30	2.16
	22	0.32	n.d.	0.54	0.54	n.d.	n.d.	n.d.	n.d.
Fat	2	n.d.	n.d.	3.43	4.46	n.d.	n.d.	15.8	18.55
	22	n.d.	-	0.40	-	0.36	-	8.90	-
Carcass	2	9.00	6.25	11.4	10.3	2.30	3.95	14.8	55.2
	22	2.16	1.14	2.85	0.85	0.22	1.21	5.15	2.16

*n.d. = not detectable; 1) 3,5-dichloro-2-methoxy-4-hydroxy-6-(dichloromethyl)pyridine; 2) 3,5-dichloro-2,4-dimethoxy-6-(dichloromethyl)pyridine.

5

Differences in metabolite distribution among different tissues have been observed in rats. O'Reilly et al., Clin. Cancer Res., 2, 541-548, March 1996. The differences demonstrate that penclomedine but not its metabolite is detectable in the nervous system during the time period of neurologic symptoms.

EXAMPLE 6

This Example illustrates the distribution of penclomedine and 4-demethylpenclomedine in human plasma during a 5 day treatment schedule using penclomedine.

Plasma levels of the metabolite and penclomedine were determined in 9 patients during the 5 day treatment schedule. After an initial 1 hr i.v. infusion of

penclomedine (315 mg/m^2), drug levels rapidly declined in an apparently biexponential fashion as shown in Figure 1 for a single individual. Demethylpenclomedine plasma levels were equal to penclomedine plasma levels by 10 min after end of

5 the infusion and rose to $9.5 \text{ } \mu\text{M}$ by 7 hrs. In five day pre-infusion and post-infusion plasma samples obtained from the same patient penclomedine was barely detectable in pre-infusion samples and were always less than μM in the post-infusion samples (see Figure 2). The ratio of

10 demethylpenclomedine to penclomedine in the post-infusion plasma sample obtained on day 1 of treatment was approximately 1:1; however, impressive accumulation of demethylpenclomedine was noted during the five day treatment schedule and by the final day this ratio was

15 often greater than 10. This metabolite persisted in the plasma for an extended period of time, producing at five days plasma exposures of the metabolite nearly 400 times that of the parent drug, as calculated by the area under the concentration-time curve. A summary of post-infusion

20 demethylpenclomedine plasma levels determined on each day of treatment for 9 patients is set forth in Table 4. While there was an insufficient number of patients at each dose level to allow statistical analysis of the means, accumulation of metabolite was observed in all 9 patients

25 over the 5 day treatment.

Table 4. Accumulation of demethylpenclomedine in patients during a 5 day treatment schedule with penclomedine

5

Patient Number	Dose (mg/m ²)	Treatment Day				
		1	2	3	4	5
1	90	1.1	5.1	4.0	8.2	-
2	135	1.6	5.1	6.1	11.5	22.4
3	135	0.6	4.8	7.0	5.7	19.0
4	180	8.2	-	17.9	38.1	51.7
5	180	2.7	7.7	16.1	26.7	42.6
6	236	1.2	7.9	19.1	28.7	35.4
7	236	0.9	6.7	17.6	31.3	32.2
8	315	1.5	5.9	14.2	15.6	25.6
9	315	3.8	17.9	29.1	41.3	50.0

All of the references cited herein including the patent and publications are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon the preferred embodiment, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiment may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. Substantially pure 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine or an acid addition
5 salt thereof.
2. A pharmaceutical composition comprising 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine
or an acid addition salt thereof and a pharmaceutically
10 acceptable carrier.
3. A method of treating cancer in a mammal
comprising administering to said mammal a cancer treatment
amount of 3,5-dichloro-2-methoxy-4-hydroxy-6-
15 (trichloromethyl)pyridine or an acid addition salt thereof.
4. The method of claim 3, wherein said cancer is
selected from the group consisting of a mammary tumor, a
brain tumor, a colon tumor, a renal tumor, an ovarian
20 tumor, neuroblastoma, and retinoblastoma.
5. The method of claim 4, wherein said cancer is
selected from the group consisting of a mammary tumor, a
colon tumor, and a renal tumor.
25
6. The method of any of claims 3-5, wherein said
treatment amount is from about 5 mg/kg to about 200 mg/kg
of the body weight of said mammal.
- 30 7. The method of claim 6, wherein said treatment
amount is from about 5 mg/kg to about 100 mg/kg of the body
weight of said mammal.
8. The method of any of claims 3-7, wherein said
35 treatment is carried out over a period of from one day to
about 24 months.

9. The method of any of claims 3-8, further comprising administering a chemotherapeutic agent other than 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine.

10. The method of claim 9, wherein said further chemotherapeutic agent is selected from the group consisting of alkylating agents, hormonal agents, antimetabolites, natural products, and miscellaneous agents.

11. The method of claim 10, wherein said further chemotherapeutic agent is an alkylating agent.

12. The method of claim 11, wherein said alkylating agent is selected from the group consisting of carmustine, chlorambucil, cisplatin, lomustine, cyclophosphamide, melphalan, mechlorethamine, procarbazine, thiotepa, uracil mustard, triethylenemelamine, busulfan, pipobroman, streptozocin, ifosfamide, dacarbazine, carboplatin, and hexamethylmelamine.

13. The method of claim 12, wherein said further chemotherapeutic agent is an antimetabolite.

14. The method of claim 13, wherein said antimetabolite is selected from the group consisting of cytosine arabinoside, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, thioguanine, floxuridine, fludarabine, cadribine, and L-asparaginase.

15. The method of claim 10, wherein said chemotherapeutic agent is a hormonal agent.

16. The method of claim 15, wherein said hormonal agent is tamoxifen.

17. The method of claim 10, wherein said chemotherapeutic agent is a miscellaneous agent.

5 18. The method of claim 17, wherein said miscellaneous agent is selected from the group consisting of mitotane, mitoxantrone, vinblastine, and levamisole.

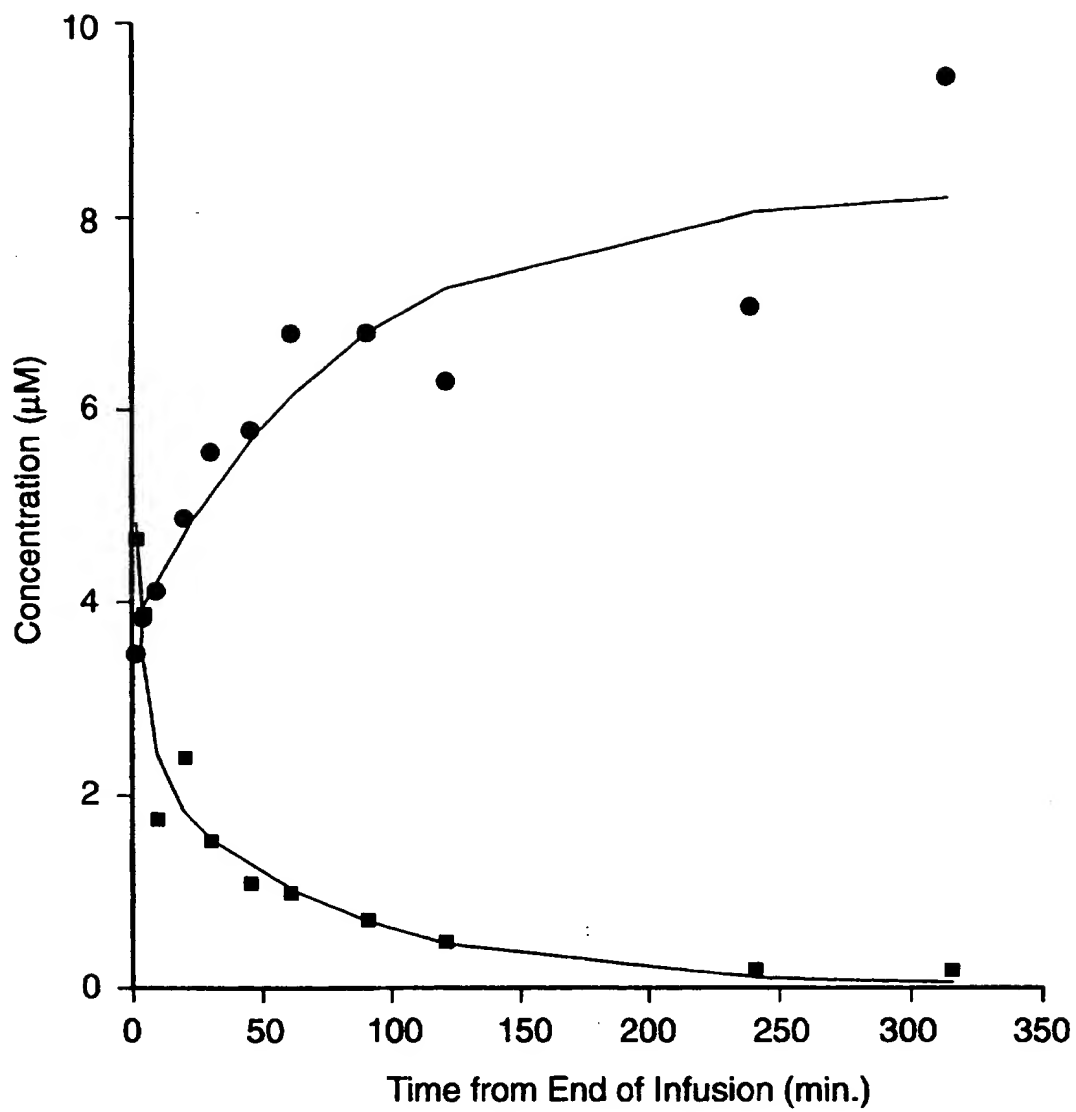
10 19. The method of claim 10, wherein said chemotherapeutic agent is a natural product.

20. The method of claim 19, wherein said natural product is selected from the group consisting of actinomycin D, bleomycin, camptothecins, daunomycin,
15 doxorubicin, etoposide, mitomycin C, paclitaxel, taxotere, teniposide, vincristine, vinorelbine, mithramycin, idarubicin, plicamycin, and deoxycoformycin.

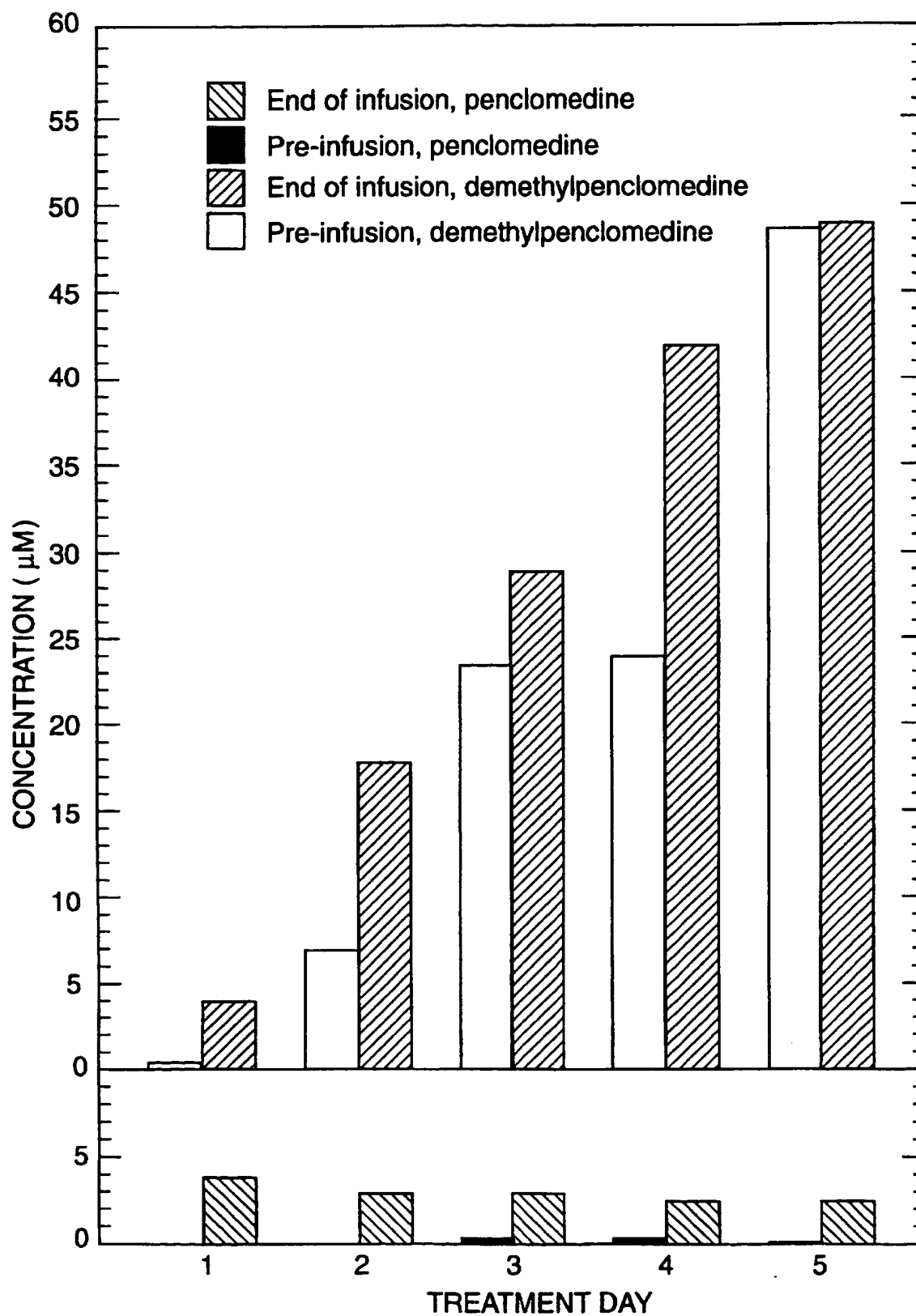
20 21. The method of any of claims 3-20, wherein said compound or compounds is administered orally or intravenously.

22. The method of any of claims 3-21, wherein said mammal is human.

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**FIG. 1**

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**FIG. 2**

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09428

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D213/69 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	W.R. WAUD ET AL.: "4-Demethylpenclofedine, an antitumor-active, potentially nonneurotoxic metabolite of penclofedine" CANCER RESEARCH, vol. 57, no. 5, 1 March 1997, MD US, pages 815-817, XP002039952 see the whole document ---	1-22
X	N.R. HARTMAN ET AL.: "Murine and human in vivo penclofedine metabolism" CLINICAL CANCER RESEARCH, vol. 2, no. 6, June 1996, ICAN ASSOCIATION FOR CANCER RESEARCH US, pages 953-962, XP002039953 see the whole document --- -/-	1-3

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

29 September 1997

Date of mailing of the international search report

08.10.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Bosma, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/09428

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. O'REILLY ET AL.: "Tissue and tumor distribution of 14C-penclofedine in rats" CLINICAL CANCER RESEARCH, vol. 2, no. 3, March 1996, ICAN ASSOCIATION FOR CANCER RESEARCH US, pages 541-548, XP002039954 see the whole document ---	1
A	US 4 717 726 A (TOBOL HELEN K) 5 January 1988 cited in the application see the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/09428

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 3-22
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/09428

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4717726 A	05-01-88	CA 1314049 A	02-03-93
		DE 3788693 D	17-02-94
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		EP 0260524 A	23-03-88
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		IE 62519 B	08-02-95
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